# Field Resistance to *Cronartium ribicola* in Full-Sib Families of *Pinus monticola* in Oregon

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**Abstract**—Two field sites were established between 1968 and 1974 using canker-free western white pine seedlings from full-sib families previously inoculated with white pine blister rust (*Cronartium ribicola*) at Dorena Genetic Resource Center. Many individuals planted on these sites had been identified as the resistant segregants for a major gene for resistance (Cr2). However, a strain of rust with specific virulence (vcr2) to this gene has been found at high frequency at and near these sites. In 1997, 27 and 92 families had surviving individuals at Blodgett Creek (BC) and Grass Creek (GC), respectively. Most of the trees on both sites were infected (99.1 percent at BC; 92.5 percent at GC). Despite heavy incidence of infection, there was striking variation in its intensity. Individual trees ranged from 0 to more than 200 cankers, and families also varied dramatically. Many of the trees at both sites continue to grow well, despite heavy infection. Wide variation in infection frequency and survival among and within families on these sites demonstrates that even the earliest selections from the program possess mechanisms of resistance other than Cr2.

**Key words**: white pine blister rust, field resistance, western white pine, virulence

## Introduction

Since its introduction to western North America near Vancouver, B.C. in 1910 (Mielke 1943), white pine blister rust (*Cronartium ribicola* J. C. Fisch.) has caused widespread damage and mortality to western white pine (*Pinus monticola* Dougl. ex D. Donn) and other five-needle pines. In the mid-1950s, the USDA Forest Service began an operational breeding program for blister rust resistance in Oregon and Washington (USDA Forest Service Region 6) to produce seedlings of western white pine for reforestation. The development of resistant populations of western white pine through breeding was seen as the best avenue for re-establishing this species. The early program included tree selection in the field, controlled pollination of selections, and artificial inoculation of the progeny.

Many of the early field selections for which progeny tests showed the most dramatic genetic resistance came from candidate trees in two areas in Oregon. The area with the highest frequency of canker-free trees was a natural second-growth stand dating from approximately 1920 in the Champion Mine area on the Cottage Grove Ranger District of the Umpqua National Forest in the Western Cascade Range. The second source of highly resistant parent trees was a plantation established with seedlings of unknown origin between 1916 and 1935 in the Bear Pass area on the Sweet Home Ranger District of the neighboring Willamette National Forest.

Survival of the parent trees at Champion Mine and Bear Pass after repeated natural epidemics was due primarily to a single dominant gene (Cr2) for resistance (Kinloch and others 1999). However, a new strain of rust appeared in the Champion Mine area around 1970 (McDonald and others 1984). Trees formerly free of infection became heavily infected. By 1994, all resistant parent trees in the Champion Mine area were dead from rust. Many of the resistant trees in the Bear Pass area still show no infection even though a low frequency of the virulent strain (vcr2) has recently been detected in this area (unpublished data).

Few long-term plantings (25 years or more) have tracked occurrence of blister rust in individual resistant families of western white pine. The plantings at Blodgett Creek (BC) and Grass Creek (GC) were primarily established with canker-free survivors of full-sib families after artificial inoculations (geographic origins are indicated in fig. 1). Soon after planting BC and GC, budgetary constraints and personnel departures resulted in their virtual abandonment. In 1996 and 1997 BC and GC were remonumented and assessed for survival, growth, and incidence of blister rust. This paper reports on the status of these two plantings following 23 to 29 years of exposure to blister rust.

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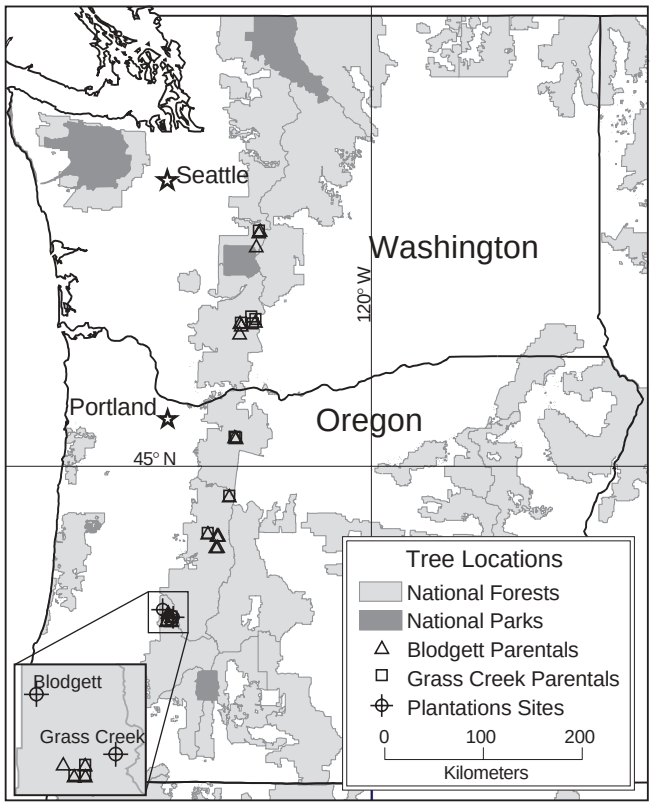



**Figure 1**—Geographic distribution of western white pine parent trees represented at experimental plantings at Blodgett Creek and Grass Creek. Insert represents plantations in Champion Mine area.

## Material and Methods

The BC and GC plantings were established between 1968 and 1974 with family row plots or blocks consisting primarily of healthy, canker-free seedlings from families that had been artificially inoculated and assessed for blister rust infection in the Region 6 program (table 1). The seedling families had been inoculated two to three times and assessed for stem symptoms. The seedlings planted at BC were from two different sowings: 1968 and 1969. Seedlings from both sowings were artificially inoculated two times with *C. ribicola* by suspending *Ribes* sp. leaves bearing mature telia over them in a moist enclosure at ambient temperatures. Seedlings planted at GC from sowings in 1959, 1962, 1963, and 1964 were artificially inoculated three times, and the seedlings at GC from the 1965 and 1966 sowings were inoculated twice. Seedlings were held for several years after the last inoculation, and age at planting varied. The number of trees planted per family varied depending upon results from the artificial inoculation. A few noninoculated families were also included.

The Blodgett Creek site is on a low elevation, relatively flat bench area and appears to have a higher exposure to rust than the Grass Creek site, which is on a steep, high elevation, south-facing slope. Both sites are within 10 miles of the Champion Mine area, where many of the parents originated, and where a strain of rust virulent to Cr2 was first identified (McDonald and others 1984, Kinloch and others 1999).

A total of 62 families (1,726 seedlings total) from 65 parents with one to 90 trees per family were planted at BC in late 1973 and early 1974. A total of 120 families (3,751 seedlings total) from 82 parents with one to 180 trees per family were represented in three plantings (1968, 1970, and 1972) at GC (table 1). The majority of families at both sites were full-sibs from controlled crosses made among 120 ortets in natural stands. The parents represented at BC came from four National Forests: Umpqua, Willamette, Mt. Hood, and Mt. Baker-Snoqualmie (fig. 1); parents at GC were predominantly from the Umpqua, Willamette, Mt. Hood, and Mt. Baker-Snoqualmie, with a few selections from BLM lands and an unknown source from Idaho.

Data on survival after first growing season were available for all plantings at the BC site and for the first (1968) planting at GC (table 2). After extensive remonumentation of each site in 1996, every identifiable tree (living and dead) was assessed for survival, diameter, and frequency and location (bole versus branch) of rust infection. When possible, the presence of cankers was determined for dead trees. Frequency of infections was assessed using a scale of 0 to 6 that was geometric, rather than arithmetic, in which each

**Table 1**—Establishment and background information for two plantings of western white pine in Oregon.

| | Blodgett Creek | Grass Creek |
|---|---|---|
| Latitude | 43.678° N | 43.601° N |
| Longitude | 122.718° W | 122.580° W |
| Elevation | 2250 ft (690 m) | 3800 ft (1160 m) |
| Aspect | Southwest | South |
| Topography | Mostly flat with some areas sloped 5-35% | Slopes 5-45% with several flat bench areas |
| Distance from Champion Mine (km) | ~8 miles (12.8 km) NW | ~3 miles (~4.8 km) ENE |
| Year(s) Established | 1973, 1974 | 1968, 1970, 1972 |
| Number of Families Planted | 62 | 120 |
| Total Number of Trees Planted | 1726 | 3751 |
| Families Surviving in 1997 | 27 | 92 |
| Trees Remaining in 1997 | 404 | 1579 |

**Table 2**—Summary of mean survival, growth and rust status of two plantings of western white pine in Oregon.

| | Blodgett Creek | Grass Creek |
|---|---|---|
| First year survival | 41.2% | n/a |
| Total survival in 1997 | 332 trees (19.2%) | 974 trees (26.0%) |
| Survival in 1997 as % of first year survival | 46.7% | 41.1% (1968 planting)<br>n/a (1970 and 1972 plantings) |
| Identifiable trees in 1997 | 404 total<br>313 healthy (77.5%)<br>19 sick or dying (4.7%)<br>33 dead <5 years (8.2%)<br>39 dead >5 years (9.7%) | 1579 total<br>755 healthy (47.8%)<br>219 sick or dying (13.9%)<br>190 dead <5 years (12.0%)<br>395 dead >5 years (25.0%)<br>20 dead, rust status unknown (1.3%) |
| Mean diameter | 19.9 cm | 16.3 cm (overall)<br>17.9 cm (1968 planting)<br>18.3 cm (1970 planting)<br>15.2 cm (1972 planting) |
| Mean canker class per tree[a] | 4.33 | 3.60 (overall)<br>4.70 (1968 planting)<br>3.09 (1970 planting)<br>3.30 (1972 planting) |
| Range of family mean canker class | 1.67-5.60 | 0-6 |
| Canker-free trees | 3 (0.4%) | 73 (6.3%) |

[a] Frequency of infection was assessed for each tree using a scale of 0 to 6. Trees in class 0 had 0 cankers; class 1, 1-3; class 2, 4-9; class 3, 10-21; class 4, 22-50; class 5, 51-100; class 6, >100.

succeeding class interval was approximately double that of the preceding class interval. Trees in canker class (CCL) 0 had no cankers; class 1 was (arbitrarily) set at 1 to 3 cankers; class 2, 4 to 9; class 3, 10 to 21; class 4, 22 to 50; class 5, 51 to 100; class 6, greater than 100. Because of the long intervals between establishment and assessment, lack of a replicated design, and nonscalar measurement of cankers/tree, no formal statistical analyses were possible.

## Results

The 1997 survey of the two sites indicated that 99.1 and 92.5 percent of the living trees have blister rust infections at BC and at GC, respectively. The mean CCL per living tree was 4.33 at BC (approximately 62 cankers) and 3.60 at GC (approximately 42 cankers) (table 2). Family mean CCL ranged from 1.67 to 5.60 at BC and from 0 to 6 at GC (fig. 2), but very few families were in CCL 2 or lower (less than 10 cankers). The number of canker-free trees was greater at GC (73) than at BC (3) (table 2).

Mean diameter (at 1.3 m) of surviving trees was 19.9 cm and 16.3 cm at BC and GC, respectively (table 2). Overall survival (including mortality within the first year following planting) was 19.2 percent at BC and 26 percent at GC. Survival varied by family (fig. 3). Some of the families with the highest survival (greater than 50 percent) differed dramatically in numbers of cankers per tree; this is also true for trees within families (fig. 4a – d for examples). When first-year mortality is excluded, survival was 46.7 percent at BC and 41.1 percent for the 1968 planting at GC (table 2).

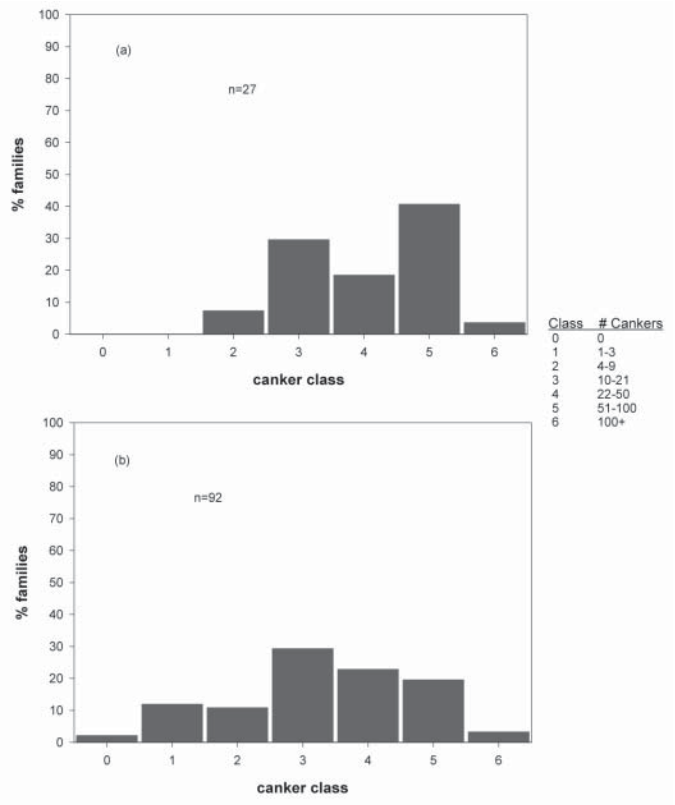



**Figure 2**—Distribution of family mean canker class (based on numbers/tree of individual trees) of 1997 survivors at (a) Blodgett and (b) Grass Creek.

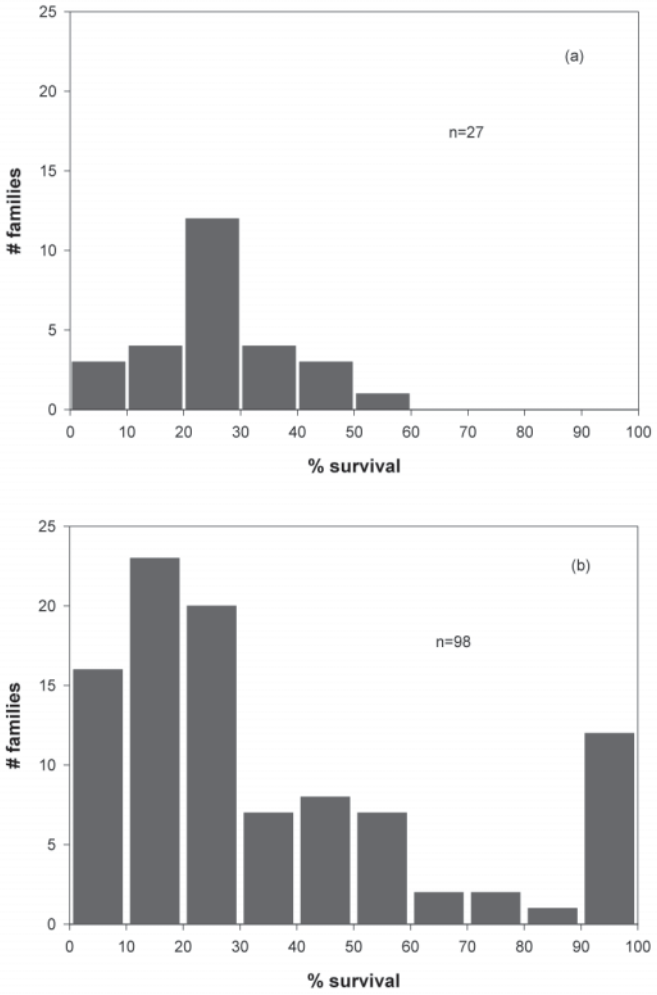



**Figure 3**—Distribution of family means for percent survival from establishment at (a) Blodgett and (b) Grass Creek

Recent mortality was greater at GC (38.3 percent) than at BC (17.9 percent) (table 2).

Atropellis canker (caused by *Atropellis* sp.) was present on trees at both sites (14 trees at BC and 189 trees at GC); some families at GC had more than 50 percent of living trees with *Atropellis*. Care was taken to distinguish this disease from white pine blister rust.

## Blodgett Creek

In 1997, 332 of the 404 identifiable trees (82 percent) were still alive, with 27 of the original 62 families still represented (with one to 31 surviving trees per family). Survival from time of planting for these 27 families was low to moderate (fig. 3a) but higher if first-year mortality was excluded. Excluding first-year mortality, survival varied from 18.8 to 85.7 percent, with an overall mean of 46.7 percent. Survival in the past 10 to 15 years was generally high for all families. Of the 72 dead trees, 33 appeared to have died within the previous 5 years (table 2).

The majority of living trees had more than 22 cankers (CCL greater than 3), and more than 20 percent of the trees had over 100 cankers (fig. 5a). On a family mean basis, the number of cankers per tree varied from five to more than 100 (fig. 2). Only three trees were canker-free on this site. Of the living trees, 73 percent had both bole cankers and branch cankers, and no trees had only bole cankers. A small percentage (5.1) of trees had only branch cankers that were relatively new (less than 5 years old), while most trees (82.5 percent) had both recent and old branch cankers. Only six trees had all cankers dead or inactive, and these were also the six trees with the fewest cankers. The two families largest in diameter had among the fewest cankers (fig. 6a).

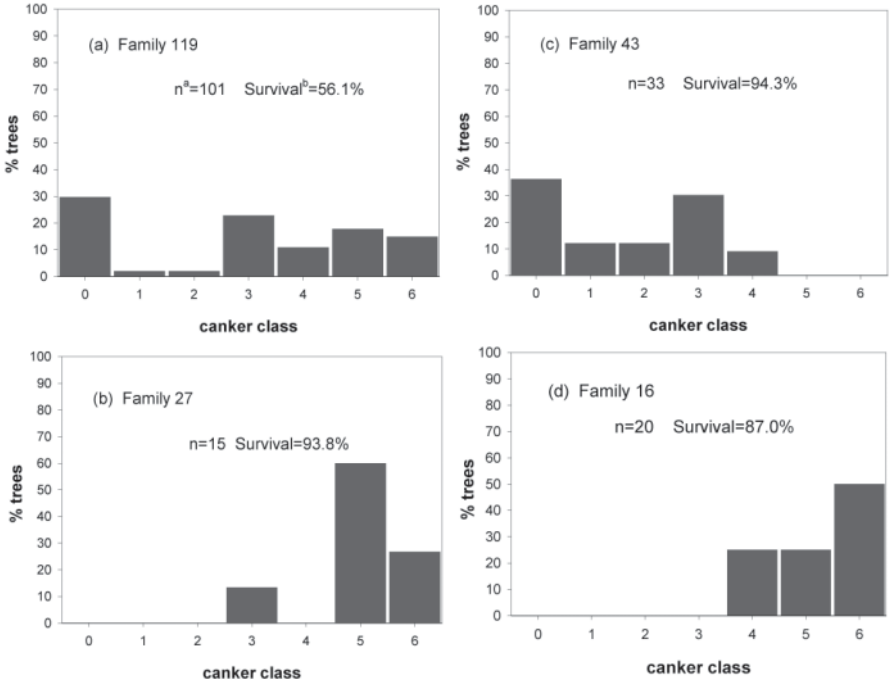
## Grass Creek

In 1997, 974 of 1579 remonumented trees were still alive, and 98 of the 120 families were still represented by identifiable living and dead trees (with one to 101 trees per family still alive). Survival from time of planting was only 26.0 percent, but some families had 100 percent survival (fig. 3b); survival in the past 10 to 15 years varied widely by family and somewhat by planting year but was high for many families. Nearly one-third (190 of the 605 trees) of the mortality recorded in 1997 appears to have occurred within the previous 5 years (table 2).

Trees in the 1968 planting averaged more than twice the number of cankers as the 1970 and 1972 plantings (table 2 and fig. 5b-d). Over 50 percent of trees in the 1968 planting had more than 50 cankers (CCL 5 and 6), while less than 15 percent of the trees in any of the three plantings were canker-free (fig. 5b-5d). Most families averaged CCL 3 or higher (minimum of 10 cankers/tree) (fig. 2b). Of the living trees, 63 percent had both bole and branch cankers, while only two trees had bole cankers only. Few trees (64) had only branch cankers that were relatively recent; most had old and recent branch cankers (82.2 percent). All cankers appeared to be dead or inactive on 15 trees. Overall, there was no strong relationship between diameter and number of cankers (fig. 6b).

A total of 73 trees, coming from 21 different families, at this site were canker-free, with most of these being in the 1970 and 1972 plantings (fig. 5b-d). These families had from one to 30 trees canker-free, and where more than 15 trees had been planted, the family mean percentage of canker-free trees was generally low (less than 10 percent). Family 103 (18034-374 x 18034-391), which originally had only three trees planted, still had 100 percent survival, and no cankers were apparent in 1997. Of six trees planted in Family 117 (06020-501 x 06020-511), the two survivors were canker-free. Family 43 (18034-395 x 18035-386) had 33 of the original 35 (94.3 percent) trees planted surviving in 1997, and 12 of these (36.3 percent) were canker-free (fig. 4c). Follow-up visits (after 1997) detected a canker in one of the trees in Family 103, and branch cankers are also present on all of the formerly canker-free trees in Family 43.

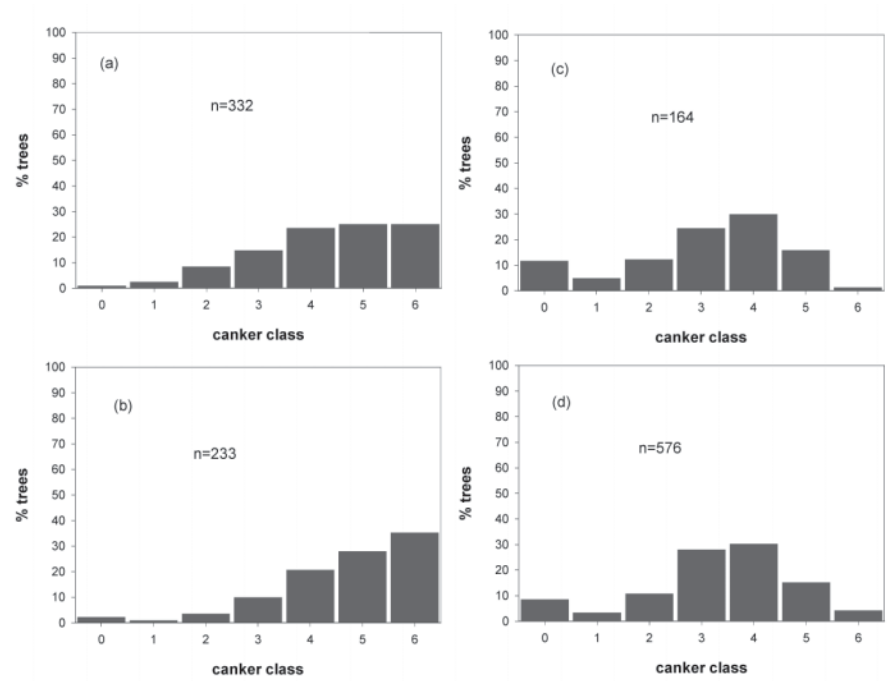
One parent, 15040-836, was involved in six of the eight crosses with highest survival; overall it was used in 18 crosses at GC. GC Family 16 (15045-835 x 15045-836) had high survival despite the presence of many cankers (fig. 4d). Another interesting parent is 06020-511 from Mt. Hood National Forest. At BC, 06020-511 was the female parent in




[a] n = number of trees living in 1997
[b] Survival from time of planting

| Class | # Cankers |
|---|---|
| 0 | 0 |
| 1 | 1-3 |
| 2 | 4-9 |
| 3 | 10-21 |
| 4 | 22-50 |
| 5 | 51-100 |
| 6 | 100+ |

**Figure 4**—Canker class distribution (1997 assessment) of living trees for two relatively resistant (a, c) and two susceptible (b, d) families at Grass Creek.




| Class | # Cankers |
|---|---|
| 0 | 0 |
| 1 | 1-3 |
| 2 | 4-9 |
| 3 | 10-21 |
| 4 | 22-50 |
| 5 | 51-100 |
| 6 | 100+ |

**Figure 5**—Canker class distribution of trees alive in 1997 at (a) Blodgett plantings established in 1973-1974 and Grass Creek plantings established in (b) 1968, (c) 1970 and (d) 1972.

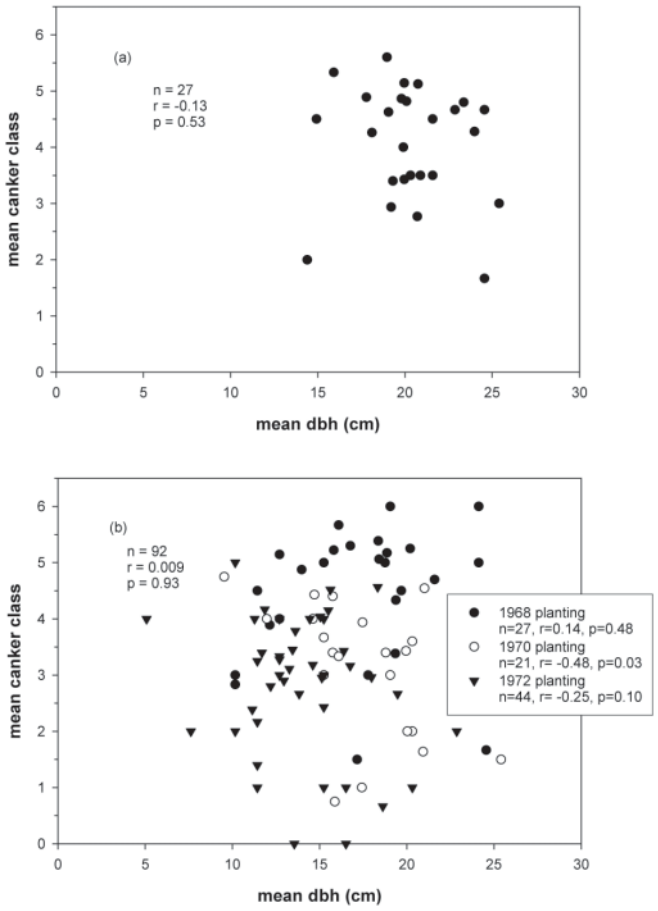



**Figure 6**—Family mean canker class per living tree versus diameter (dbh) at (a) Blodgett and (b) Grass Creek.

two crosses and the male parent in two crosses. At GC, 06020-511 was represented in three families, twice as the male parent and once as an apparent self (Family 119). GC Family 119 had 180 seedlings planted; in the 1997 inventory, 101 of these trees were still alive, of which 30 (29.7 percent) were canker-free (fig. 4a). Many other trees in this family appeared vigorous despite having large, old bole cankers. Although only one of the two families at BC with 06020-511 as the female parent had surviving trees in 1997, this family had the lowest mean canker class (1.67) and the second largest mean DBH (24.6 cm) (fig. 6a).

## Discussion

The high level of rust infection at these sites was somewhat unexpected since most of the seedlings were canker-free after heavy artificial inoculation. However, recent investigations make it apparent that the main cause of the high level of infection is the presence of a virulent strain of rust (Kinloch and others 1999, Kinloch and Dupper 2002). Many of the parents represented in the planting are now known to carry a specific gene for resistance (Cr2) that is neutralized by a corresponding gene for virulence (vcr2) in the pathogen in a gene-for-gene relationship (Kinloch and others 1999, Kinloch and Dupper 2002). High inoculum loads and intense selection pressure caused a sudden and dramatic increase in vcr2 in these plantations.

The families planted on these sites were some of the first selections made in the Region 6 program (Kinloch and others 1999). Despite a relatively narrow genetic base among these early families, wide variation in infection frequency and survival of trees on these sites indicates that non-Cr2 resistance is also present. This became apparent only after Cr2 was neutralized by vcr2, thereby unmasking independent, unrelated mechanisms. These mechanisms are forms of partial resistance that reduce infection rate or allow the tree to survive after infection. Similar results have been reported for progenies of sugar pine (*P. lambertiana* Dougl.) with a different major gene for resistance to blister rust (Cr1). After many years exposure to a strain of rust (vcr1) with specific virulence to Cr1, most of the sugar pine trees were killed, but a significant number exhibited mechanisms of partial resistance unrelated to Cr1 that enabled them to survive and in many cases heal (Kinloch and Davis 1996). One of these mechanisms included infection frequency differences of a similar magnitude to those observed at BC and GC.

Wave years of infection occurred frequently at both sites after plantation establishment. Many trees have dozens or more cankers but are still showing vigorous growth. Some individuals have large bole cankers that have been present for over two decades (an indication of tolerance), for example, family '119' at GC. One reason many of these trees are still alive is that most of the cankers at these two sites are branch cankers more than 0.5 m from the main stem and so are unlikely to reach the bole. These two plantings will continue to be monitored to see if the cumulative blister rust impacts over time lead to mortality directly (bole girdling) or indirectly (crown thinning or predisposition to other agents such as bark beetles), and whether the resistance is durable. From a silvicultural point of view, it is encouraging to see trees that still thrive after nearly 30 years of intense white pine blister rust exposure.

Due to the gap in data collection between trial establishment and the 1997 assessment as well as the substantial first-year postplanting mortality, it is not possible to clearly delineate all of the resistance mechanisms that might be present. Although the physiological basis and inheritance of additional mechanisms is unknown, at least several phenotypes have been documented: canker-free, low infection frequency (fewer than average cankers present), tolerance (vigorous tree with large bole cankers), and bark reaction (healed or inactive cankers present). The gap in data limits some of the specific information that could have been garnered for each family, such as for small, ephemeral bark reactions.

These two sites represent some of the earliest field plantings of the first resistant trees produced by the Region 6 western white pine blister rust resistance program. Although only canker-free seedlings were deployed, survival of these trees over nearly 30 years of exposure to a virulent strain of rust can be attributed to partial resistance mechanisms. These mechanisms may provide the foundation for establishing durable resistance in future generations of western white pine. Collections of wind-pollinated lots from several trees at BC and GC have been made, and these seedlots have been included in recent rust-screening trials.

Since these two sites were established, progeny of thousands of parent trees have been screened for a more diverse set of putative resistance mechanisms (Sniezko 1996), and replicated field validation tests have been established (Sniezko and others this proceedings, Sniezko and others 2000). Modifications to the operational screening program continue as new information on resistance mechanisms becomes available. Resistant seed from the breeding program will be used to help restore and maintain western white pine as a valuable component of the forest ecosystems in Oregon and Washington.

## Acknowledgments

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